International Journal of Agricultural Science and Research (IJASR)
ISSN(P): 2250-0057; ISSN(E): 2321-0087
Vol. 6, Issue 2, Apr 2016, 139-144

TRANS STELLAR
•Journal Publications •Research Consultancy

EFFECT OF GENOTYPE, PRE COLD TREATMENT AND MEDIA ON ANDROGENESIS OF RICE (*ORYZA SATIVA L.*)

ARJUNAPPA H M[1], SATEESH KUMAR P[2], RAJESH B[3] & PREMALATHA D[4]

[1, 2, 3] *Department of Biotechnology, Nuziveedu Seeds Limited, Hyderabad, Telangana, India*

[1]*Department of Biotechnology, Jawaharlal Nehru Technological University, Hyderabad, Telangana, India*

[4]*Department of Biotechnology, St Martins Engineering College, Hyderabad, Telangana, India*

ABSTRACT

In vitro production of double haploid plants through anther culture technique is an important tool for rapid production of new rice varieties. 8F1, 1F2 and 1 F3 genotypes of rice were studied for the effect of genotype, media and cold pre treatment on androgenesis. The panicles were pre cold treated at 9°C and later anthers were inoculated in N6 basal medium containing 1mg/L 2,4-Dichlorophenoxy acetic acid, 1mg/L Naphthalene acetic acid(P2) and 1mg/L 2,4-Dichlorophenoxy acetic acid, 1mg/L Naphthalene acetic acid and 0.5mg/L kinetin(P9). The callus developed was transferred to MS medium with 2.0 mg/L Kinetin+0.5mg/L Naphthalene acetic acid for regeneration. Genotype NPF1-407 showed maximum callus frequency in P9 medium and in 10 days cold treatment. Highest plant frequency of 100% was observed in NPF1-403 genotype and in 11 Days cold treatment. Thus showing that 11 Days cold pre treatment in P9 medium showed maximum plant frequency, based on total number of plants produced for total anthers inoculated showed better response in P9 medium 10 days cold treatment.

KEYWORDS: Androgenesis, Anther Culture, Callus, Double Haploid, Flow Cytometry, Pre Cold Treatment

Received: Feb 24, 2016; **Accepted:** Mar 01, 2016; **Published:** Mar 04, 2016; **Paper Id.:** IJASRAPR201619

Original Article

INTRODUCTION

Rice is a staple food for a large population and is of economic importance. Khush and Virmani, 1996 found that traditional crop breeding is a time consuming procedure when compared to double haploid technology which fastens crop improvement and introduction of new breeds. Homozygous plant lines are essential for speedy crop improvement as stated by Basu et al., 2011. Androgenesis of heterozygous F_1 hybrid brings in homozygous plant lines in one generation by chemical induction or spontaneous production. These plants are referred as double haploids and high yielding, disease resistant, Improved quality varieties are derived through these double haploids as stated by Hu, 1985; Chung, 1992.Gioi and Tuan, 2002 found that decreased callusing, low regeneration potential, genotype dependency, incidence of haploid plants are the important drawback of androgenesis. Genotype, pre treatment, culture media composition influences callus induction and plant regeneration. Hence genotype contributes to the in vitro response of cereal tissues culture as stated by Hartmann et al., 1989.

The aim of our study was to develop efficient androgenesis protocol for production of double haploids for different Indica Rice genotypes to shorten the breeding cycle and fix agronomic traits.

MATERILAS AND METHODS

NSL breeding lines and the hybrids of Indica subspecies (NSLF1 910, 2035, 388, 389, 403, 404, 407,413

NSL F2 201 and NSL F3 394) were used in the study. Anther donor plants were grown at Nuziveedu Seeds Limited field in Hyderabad. Panicles were collected from primary and secondary tillers during morning hours when the panicles were still enclosed within the leaf sheath with distance between auricle and next subtending leaf was 7-10 cm.

Pre Cold Treatment

Harvested panicles were cleaned and wrapped in moist tissue paper and were again wrapped in aluminum foil and cold treated at 9°C for 10 and 11days.

Surface Sterilization

Florets with anthers containing microspores at mid to late uninucleate stage were selected and sterilized by treating with 70% ethanol for 1 minute and rinsed with sterile distilled water for 3 times.

Culture Media and Culture Conditions

Isolated anthers were inoculated on Chu N6 (Chu 1975) basal medium containing 1mg/L 2,4-Dichlorophenoxy acetic acid, 1mg/L Naphthalene acetic acid (P2), 1mg/L 2,4-Dichlorophenoxy acetic acid, 1mg/L Naphthalene acetic acid and 0.5mg/L kinetin (P9) with 50 grams per liter maltose then the media was adjusted to pH 5.8 and was solidified with 0.8% agar. The media was autoclaved at 121^0C for 20 minutes; The Cultures were kept in darkness for 4- 6 weeks at 25° C for embryogenic callus induction. When the calli was 2-3 mm in diameter, they were transferred to regeneration medium made up of MS (Murashige and Skoog.1962) Medium with 2mg/L kinetin, 0.5mg/L NAA and 30 grams per liter Sucrose. Regenerated plants were sub cultured in ½ MS medium with 0.3mg/L kinetin for shoot and root elongation. Well developed plants with roots were hardened and transplanted to the field. The ploidy levels were confirmed by chromosome count in root tip method and flow cytometry (Arjunappa H M et al. 2016). Chromosome doubling of haploid plants was induced by treating with 0.3% Colchicine.

RESULTS AND DISCUSSIONS

Production of DH plants is an important tool for crop improvement as it enables us to obtain homozygous plants directly from hybrid. Genotypes, different media composition and growth regulators, pre cold treatments are vital key factors for successful embryogenesis and plant regeneration (Figure 1-4). Mandal and Gupta in 1995 proved that genotype as the deciding factor for androgenesis, variations in media composition and different concentrations plant growth regulators are the other factors.

Effect of Genotype on Embryogenesis

In our study 8 F1, 1F2 and 1 F3 genotypes were studied for their androgenic effect, each genotypes response was different and varied due to different cold treatment and media (Table 1). NPF1-407genotype showed maximum callus frequency of 22.6% and maximum plant frequency of 100% was observed in NPF1-403 genotype respectively.

Genotype is one of the major factors influencing androgenesis and plant regeneration. Significant variation in their ability to form callus and efficiency in plant regeneration is the evidence of genotypic difference. Varied response was seen not only in species within genus but also in the cultivars of same species (Nitsch1969; Guha-Mukherjee 1973; Irikura1975; Tomes and Collins1976). Davoyan 1987 stated that callus formation and regeneration capacity were controlled by independent genes and clear resemblance of low callus formation was observed in F1 seeds which was a dominant trait in their parent, clearly showing that suppressed callus formation genes were dominant.

Table 1: Effect of Cold pre treatment, Media on Callus and Plant Frequency

Genotype	No of Anthers Inoculated	Cold Pre Treatment	Media	No of Callus Produced	Callus Frequency	No of Green Plants Produced	Green Plant Frequency
NPF1-910	1016	10 DCT	P2	60	5.9%	21	35.0%
	1842		P9	53	2.9%	25	47.2%
	1015	11 DCT	P2	63	6.2%	12	19.0%
	711		P9	9	1.3%	3	33.3%
NPF1-2035	1171	10 DCT	P2	88	7.5%	2	2.3%
	1820		P9	205	11.3%	9	4.4%
	666	11 DCT	P2	37	5.6%	5	13.5%
	1104		P9	59	5.3%	22	37.3%
NPF1-388	1004	10 DCT	P2	88	8.8%	12	13.6%
	1331		P9	300	22.5%	73	24.3%
	562	11 DCT	P2	56	10.0%	15	26.8%
	605		P9	83	13.7%	19	22.9%
NPF1-389	443	10 DCT	P2	8	1.8%	1	12.5%
	296		P9	36	12.2%	7	19.4%
	868	11 DCT	P2	19	2.2%	4	21.1%
	802		P9	5	0.6%	4	80.0%
NPF1-403	632	10 DCT	P2	37	5.9%	19	51.4%
	102		P9	2	2.0%	0	0.0%
	1005	11 DCT	P2	10	1.0%	10	100.0%
	134		P9	4	3.0%	0	0.0%
NPF1-404	1090	10 DCT	P2	106	9.7%	0	0.0%
	745		P9	40	5.4%	25	62.5%
	851	11 DCT	P2	82	9.6%	12	14.6%
	636		P9	0	0.0%	0	0.0%
NPF1-407	1089	10 DCT	P2	18	1.7%	0	0.0%
	820		P9	185	22.6%	19	10.3%
	758	11 DCT	P2	135	17.8%	0	0.0%
	1092		P9	20	1.8%	5	25.0%
NPF1-413	851	10 DCT	P2	0	0.0%	0	0.0%
	636		P9	82	12.9%	12	14.6%
	987	11 DCT	P2	88	8.9%	12	13.6%
	868		P9	19	2.2%	4	21.1%
NPF2-201	1061	10 DCT	P2	58	5.5%	1	1.7%
	915		P9	85	9.3%	7	8.2%
	828	11 DCT	P2	117	14.1%	1	0.9%
	515		P9	12	2.3%	0	0.0%
NPF3-394	1000	10 DCT	P2	52	5.2%	12	23.1%
	436		P9	24	5.5%	12	50.0%
	315	11 DCT	P2	19	6.0%	4	21.1%
	334		P9	29	8.7%	1	3.4%

Effect of Cold Treatment on Embryogenesis

Cold treatment of panicles for 10 days and 11 days showed varied response in callus formation and plant regeneration. 10 days cold treatment showed more callus response when compared to 11days cold treatment but 11 days showed more plant regeneration frequency than 10 days cold treatment. The best pre treatment time was10 DCT as it depends upon the genetic material of plant genotypes.

The effect of cold pre treatment is an important factor for callus induction and development as stated by Sopory and Munshi 1996. The effect of chilling pre treatment is also genotype depended as reported by Datta 2005. The duration,

time of application and type of cold pre treatment differs with species or varieties and has androgenesis promontory effect in many plant species. Plant regeneration is influenced by cold treatment and high temperature may induce secondary embryo formation which lack normal regeneration capacity as stated by Sopory and Munshi 1996. During low temperature treatment few of the microspore slowly develop into embryo while most of them develop into normal pollen, this is the reason why cold pre treatment has promontory effect on embryogenesis.

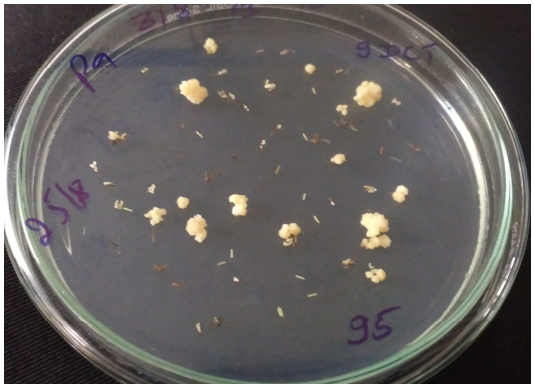

Figure 1: Initiation of Embryogenic Calli after 4 Weeks of Culture

Figure 2: Androgenic Plant Development on Regeneration Medium

Figure 3: Well Developed Androgenic Plants on Rooting Medium

Figure 4: Double Haploid Plants in Field

Effect of Media Composition on Embryogenesis

Media with different concentrations of growth regulators play an important role in callus formation. P9 medium showed significantly higher callus and plant formation frequency than P2 medium.

Plant growth regulators are investigated widely and regarded as an important factor in callus induction and plant regeneration. Different combinations and concentrations of these hormones affect the development of calli and morphogenetical growth of plant regenerant as stated by Trejo-Tapia et al, 2002. Ball 1993, stated the growth factor types, their concentration and their presence are the factors that influences embryogenesis. 2,4-Dichlorophenoxyacetic acid (2,4-D) and Naphthalene acetic acid (NAA) are commonly used synthetic auxins as these are the essential for callus induction was shown by Zhu et al..1998, but prolonged exposure and high concentration of the same 2,4-D causes chromosomal abnormalities and frequency of plant regenerants as discussed by Deambrogio and Dale, 1980; Ziauddin and Kasha, 1990.

CONCLUSIONS

Successful production double haploid in rice depends on genotypes, culture media, cold pre treatment as these are the critical factors which synergistically play an important role in embryogenesis. In our study we have found similar results. Genotypes show varying response in different media with varying concentrations of growth regulators and different pre cold treatment. The best callus frequency and plant regeneration frequency was seen at pre cold treatment at for 10 days in P9 medium.

ACKNOWLEDGEMENTS

We are thankful to Mandava Prabakar Rao, CMD, Nuziveedu Seeds Limited, Hyderabad for Funding the project and facilities provided.

REFERENCES

1. *Arjunappa H M, Sateesh Kumar P, Premalatha D. 2006.Studies on ploidy analysis and Chromosome doubling in Androgenic plants of Chilli pepper (Capsicum annuum L.). International journal of Agriculture innovations and Research 4: 627-674*
2. *Ball ST, Zhou H, Konzak CF.1993. Influence of 2, 4-D, IAA and duration of callus induction in anther cultures of spring wheat. Plant Science 90: 195-200*
3. *Basu SK, Datta M, Sharma M, Kumar A.2011. Haploid production technology in wheat and some selected higher plants. Austalian Journal of Crop Science 5(9): 1087-1093*
4. *Chu, C.C., Wang,C.C., Sun, C.S., Hu, C., Yin, K.C. and Chu, C.I. (1975), Establishment of an efficient mediwn for anther culture of rice through comparative experiments on the nitrogen sources, Sci. Sinica, 18, 659-668.*
5. *Chung GS.1992. Anther culture for rice improvement in Korea. Anther culture for rice breeds. Hangzhou, China 9-24*
6. *Datta, S.K. (2005). Androgenic haploids: Factors controlling development and its application in crop improvement. Current Science. **89** (11): 1870–1878.*
7. *Davoyan, E I. 1987.Genetic determination of the process of callus formation and induction of regenerants in tissue culture of Rice. Generika.USSR 23:303-310*
8. *Deambrogio E, Dale PJ.1980. Effects of 2,4-D on the frequency of regenerated plants in barley and on genetic variability between them. Cereal Research communications 8: 417-423.*
9. *Guha Mukhergee. S. (1973), Genotypic differences in the in vitro fonnation 0 fembryoids from rice pollen, 1. Exp. Bot., 24, 139-144.*
10. *Gioi TD, Tuan VD.2002. Effect of different media and genotypes on anther culture efficiency of F1 plants derived from crosses between IR64 and new plant type rice cultivars. Omonrice 10: 107-109*
11. *Hartmann C, Hery Y, DeBuyser J, Aubry C, Rode A.1989.Identification of new mitochondrial organization in wheat plants regenerated from somatic tissue culture. Theory and Applied Genetics 77: 169-175*
12. *Hu H.1985. Use of haploids for crop improvement in China. Genetic Manipulation Crops Newsletter 1: 11-23*
13. *Irikura Y (1975) Induction of haploid plants by anther culture in tuber bearing species and inter specific hybrids of Solanum. Potato Res 18: 133-140*

14. *Khush GS, Virmani SS.1996. Haploids in plant breeding. In: Jain SM, Datta S, Veilleux RE (Eds). In vitro haploid plant production in higher plants. Fundamental aspects and methods. Kluwer Academic Publishers. Dordrecht 1:11-33*

15. *Murashige T, Skoog F.1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiologia Plantarum 15: 473-497*

16. *Nitsch, J. P., and C. Nitsch. "Haploid plants from pollen grains." Science 163.3862 (1969): 85-87.*

17. *Sopory, S.K. and Munshi, M. (1996). Anther Culture. In: S.M. Jain, S.K. Sopory, and R.E. Veilleus (Eds.). In vitro Haploid Production in Higher Plats. Kluwer Academic Publishers, Netherland. 145–176.*

18. *Tomes,D.T. and Collins,G.B. (1976) Factors affecting haploid plant production from in vitro Anther cultures of Nicotiana species. Crop. Sci.,16. 837-840*

19. *Trejo-Tapia G, Amaya UM, Morales GS, Sanchez ADJ, Bonfil BM.2002. The effects of cold pretreatment, auxins and carbon source on anther culture of rice. Plant Cell Tissue Organ Cult 71: 41-46*

20. *Zhu DY, Sun ZX, Pan XG, Ding XH, Shen XH, Won Y, Pan H, Yin JH, Alejar MS, Torrizo LB, Datta SK.1998. Use of anther culture in hybrid rice breeding, Proceedings of the 3rd International Symposium of Hybrid Rice. 14-16 Nov. 1996. Hyderabad, India. In: Advances in Hybrid rice Technology. International Rice Research Institute, Manila, Phillipines. 21:265-281*

21. *Ziauddin A, Kasha KJ.1990. Long term callus cultures of diploid barley (Hordeum vulgare L.) II. Effect of auxins on chromosome status of cultures and regeneration of plants. Euphytica 48: 279-286.*